

DESIGNING AND FABRICATION OF A MULTI SLIDE SDS PAGE DEVICE

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Bachelor of Technology

In

Biomedical Engineering

By

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National Institute of Technology, Rourkela

CERTIFICATE

This is to certify that the project entitled, “**Designing and fabrication of a multi-chamber electrophoretic device**” submitted by **Samir Kumar Sethi** is an authentic work carried out by him under my supervision and guidance for the partial fulfillment of the requirements for the award of **Bachelor of Technology (B. Tech) Degree in Biomedical Engineering** at **National Institute of Technology, Rourkela**.

To the best of my knowledge, the matter embodied in the thesis has not been submitted to any other University/ Institute for the award of any Degree or Diploma.

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“ALL KNOWLEDGE HURTS.”

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Samir

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ABSTRACT

Currently available electrophoresis devices for SDS PAGE (Sodium dodecyl sulfate polyacrylamide gel electrophoresis) are less efficient in terms of sampling rate and the material used/assembly. Usually the systems are closed system i.e. the glass slabs that are being used in these devices are unique for a particular model in terms of shape and size and vary from model to model. Glass slab of one model cannot be used in another model. Such devices once damaged are not cost effective for reuse. For running an SDS PAGE it takes 30 minutes in mini gels and 2-3 hours for large gels such that a multi chamber device is required for simultaneous running of gels for different samples. In the current project, a slide based 3-D model of gel electrophoresis apparatus was designed in order to fasten the run time, decrease the cost and increase the through output. Briefly, a pair of microscope glass slide with spacer in place of glass slabs was used in the compartments grooved in cylindrical cartridges. Thus the running platform unlike the glass slabs is universally constant and is easily available. At the same time, a specialized multi-compartmental set up was designed to house the glass slides embedded in the cartridges with a common buffer solution running through it. Such a set up enabled the separation and analysis of different samples of proteins simultaneously. The in-house developed SDS PAGE was cost effective and the number of samples can be increased by simply increasing the number of cartridges in the system.

Keywords: Electrophoresis, SDS PAGE, Microscope glass slides, Spacer, Cartridges

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Abbreviation

| | |
|------|------------------------------------|
| SDS | Sodium dodecyl sulphate |
| PAGE | Polyacrylamide gel electrophoresis |
| BSA | Bovine serum albumin |

CHAPTER 1

INTRODUCTION

Protein separation is extremely important to separate or characterize one single protein or a few proteins from a mixture like cells, tissues etc. Protein separation is required to find different characteristics of the protein like its structure, function and its interaction with other molecules, cells etc. Also protein separation helps to isolate protein and non-protein molecules in a mixture. Isolation of proteins is usually based on protein size differences, differences in biological activity, binding affinity, physical and chemical properties. These properties are mainly divided into analytical and preparative. Analytical method is mainly used to identify a particular protein or proteins in a mixture. But preparative methods are used to produce proteins in large amount. Mainly analytical method is used for research or research related purposes whereas preparative method is used in large amount production for commercial purposes like insulin, enzyme nutritional protein production. Gel electrophoresis is an analytical method in which separation of proteins depends on the movement of charged particles in an electric field. Whereas in SDS PAGE the process only depends on protein size.

1.1 Electrophoresis

Electrophoresis is an analytical method. This method is applied for the separation and characterization of macromolecules like proteins, nucleic acids and subcellular-sized particles like viruses and small organelles. Its principle is that in a sample having charged particles when migrate in an applied electrical field and if conducted in a solution, samples are separated based on their surface net charge density [1]. In this method a charged particle experiences a force in an electric field which is directly proportional to the potential difference of the electric field (E) and net charge of the molecule (q) and inversely proportional to the distance (d) between the electrodes. Hence the force experienced by the molecule is expressed as [2]:

$$F = E q / d$$

The above force is opposed by a frictional force which is equal to the product of the frictional coefficient (f) and velocity of the particle (v). The frictional coefficient depends upon the shape and size of the molecules and the viscosity (η) of the medium.

$$F_t = 6\pi \eta r v$$

When a particle moves in a velocity v then these two forces, i.e. Force due to electric fields and due frictional force is equal, which is:

$$6\pi \eta r v = E q / d$$

$$\Rightarrow v = E q / d 6\pi r \eta \text{ (mobility)}$$

The above equation says that the mobility of a molecule moving in an electric field is proportional to the electric field strength (i.e. E/d) and inversely proportional to the frictional coefficient (i.e. Shape and size of the molecule and viscosity of the medium).

1.2 Gel Electrophoresis

Electrophoresis of macromolecules like protein, DNA, RNA has lower efficiency to separate molecules due to diffusion [3]. To achieve greater efficiency electrophoresis is carried out on semi-solid matrix like polyacrylamide or agarose gels. The gels are crosslinking polymers that are formed in aqueous medium. These crosslinking polymer forms a three dimensional network, through which these macromolecules pass through. Mainly two types of gels are used polyacrylamide and agarose gels. Agarose gels have a larger pore size than acrylamide gels hence it is generally used for larger macromolecules. Gels are formed when long polymers are cross-linked in a lattice. The cross-linked polymers left a space between them, which form pores. Higher concentration of polymer leads to smaller average pore size. The cross-linking of the acrylamide polymers results in 'pores' of a defined size. Polyacrylamide gels are formed when acrylamide monomers covalently cross-linking with bis-acrylamide with a free radical like per sulfate ($\text{SO}_4\cdot$).

Smaller molecules will be able to move through this lattice more easily than larger molecules resulting in larger molecules having a lower mobility than smaller molecules. The gel is like a molecular sieve that retains the larger molecules, while letting the smaller ones to pass through it.

Agarose gels have a non-uniform pore size, but are optimal for electrophoresis of proteins having mass larger than 200 kDa. It can also be used for the separation of DNA fragments in the range of 50 base pair to several megabases, the largest of which require specialized apparatus. Polyacrylamide gel electrophoresis (PAGE) is used for separating proteins in the range of 5 to 2,000 kDa because of uniform pore size provided by the polyacrylamide gel and SDS PAGE (discontinuous) can be used to separate proteins lie in the range of 1-100 kDa [4]. Pore size is controlled by varying the concentrations of acrylamide and bis-acrylamide powder used in preparation of gel.

1.3 SDS PAGE

Sodium dodecyl sulphate is an ionic detergent which denatures the protein molecules without breaking peptide bonds. It strongly binds to all proteins and creates a very high and constant charge to mass ratio for all denatured proteins. After treatment with SDS, irrespective of their charges all proteins acquire a high negative charge. Denaturation of protein is usually done by heating them in a buffer containing a soluble thiol reducing agent and SDS. Mercaptoethanol reduces all disulfide bonds of cysteine to free sulphydryl groups, and heating with SDS disrupts all intra-inter molecular protein interaction. This treatment results individual polypeptide chains which have an excess negative charge induced due to binding with detergent [5]. This means, coating proteins with a negatively charged detergent reduces the effects of a protein's net charge. Now, during electrophoresis the mobility strictly depends upon its size.

In SDS gel electrophoresis mobility is expressed as a relative mobility (R_f).

$R_f = \text{distance protein migrated} \div \text{gel length}$

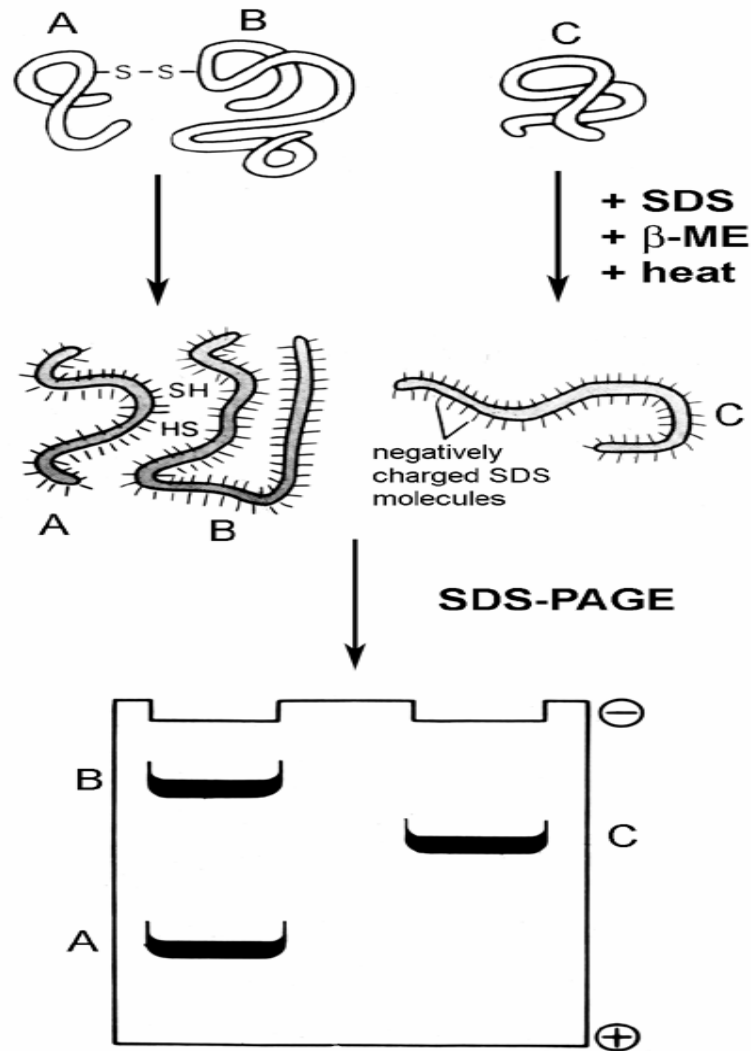


FIGURE 1.1 Denaturation of proteins by Mercaptoethanol and SDS and resulting negatively charged protein then PAGE is done on them.

The length of the gel is the migration of a substance which is not obstructed by the matrix like a small molecular weight tracking dye (for example bromophenol blue). The mobility obtained from above formula can be used to calculate the size of the protein. Then protein standards of known are used to generate a standard curve which is done by plotting the log of the molecular weight against R_f values.

CHAPTER 2

LITERATURE REVIEW

2.1 History:

In 1930s, protein electrophoresis were first developed in which sucrose and starch was used as separating media [6]. After that in the late 1950s many improvements were done, people were doing PAGE (Polymerized gel electrophoresis) in which one can control the size of the pore for better resolution. In the mid to late 1960s electrophoretic analysis became the major protein analytical tool. During this period denaturing and discontinuous stacking system was used. Then in the early 1970s commercial slab gels came into existence. During the period of the last three decades the science and technology used in electrophoretic analysis hasn't been changed but during this period there has been many improvements like cost has been lowered, easy to handle great resolution, higher reproducibility and accuracy etc. Some of these devices can resolve in a range of 5-250 KD. In the last 15 years the development of microfluidics like lab on a chip has led to automation of electrophoretic analysis of proteins.

2.2 Some currently available devices and its problems:

Some of the multi-chamber SDS PAGE devices are Protean II multi-gel casting chamber, Mini-Protean 3 multi-casting chamber, or Protean Plus multi-casting chamber. In all these models gel solution leakage is a major problem because these processes two gels resolving and stacking gels are used and we have to clean these slabs thoroughly after analyzing for the next run. And during cleaning if a very small crack appears in the corner, then it will lead to leakage. Hence the glass slabs should be handled very carefully. In these models the pair of glass slabs for holding gels is sandwiched over one another to achieve multi sample casting and running.

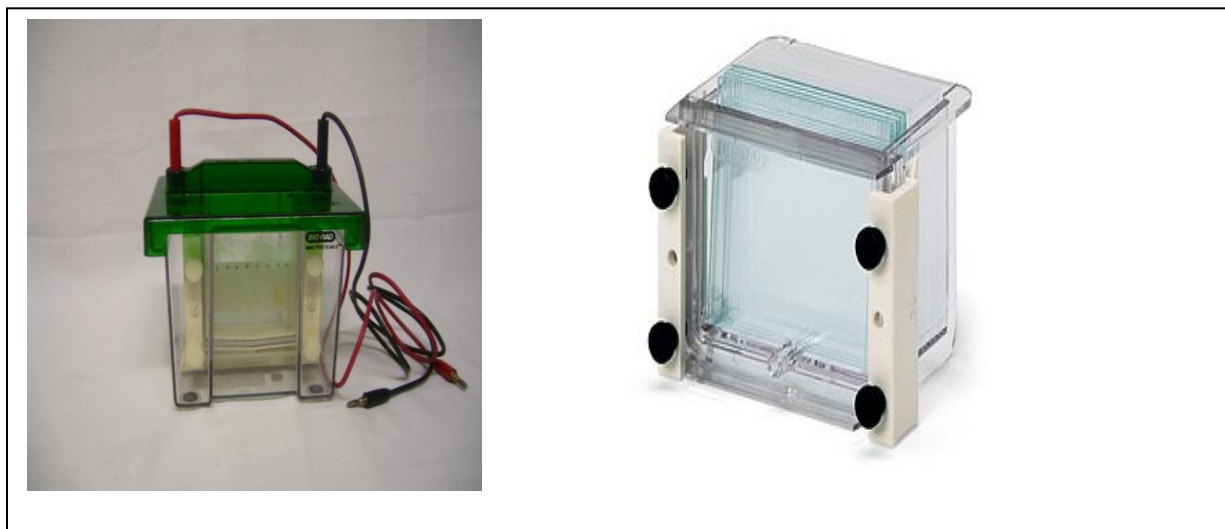


FIGURE 2.1 Mini- PROTEAN® II and 3multi-caster showing glass slabs sandwiched with one another

Then these glass plates are put inside the chamber even if there is any crack in the glass slab there will be no leakage. Also, we can paraffin film to avoid leakage. First, we have to stretch the parafilm then we have to place the films on the edges, due to tensile stress it attaches with the slab and stops leaking.

2.3 Applications:

Glass slides were used for different electrophoretic applications like for separation of serum lipoproteins an ultramicro electrophoretic technique was used using agarose gel on micro slides [7]. Here glass was used for setting the gel and was made from microscope slide, 7.5 cm x 5 cm x 1-5 mm. Two glass strips of 1 mm thick and 6 mm wide were fixed longitudinally to the top surface of any of these slides by applying epoxy resin. Another microscope slide of same dimensions is placed on the top of the sandwich so formed containing an empty space of volume 3.8 cm x 7.5 cm x 1-0 mm in size. Then the template can be placed shallow dish 5.5 cm x 8 cm. The separation between the three major lipoproteins was found to be good and there was no crescent formation of the bands while other methods like of Kalab and Martin (1968) shows the crescent formation. Reproducibility of this method was found to be good provided that the staining procedure was strictly followed.

Likewise comet assay is a sensitive and uncomplicated technique that can be used for detection of DNA damage at the level of the individual eukaryotic cell [8]. A sample of cells, derived from an in vivo test or from an in vitro cell culture subject is dispersed into individual cells and suspended in molten low-melting-point agarose at 37 °C. This mono-suspension is then cast on a microscope slide. A glass cover slip is then held at an angle and the mono-suspension applied to the point of contact between the coverslip and the slide. As the coverslip is lowered towards the slide the molten agarose spreads so that a thin layer is formed. The agarose is gelled at 4 °C and the coverslip removed. Then the agarose forms a matrix of carbohydrate fibres which encapsulate the cells, by anchoring them in place. The agarose is an osmotic-neutral; therefore solutions can penetrate through the gel and affect the cells without shifting position of cells. In an in vitro study it was found that the cells would be exposed to a test agent - typically UV light, ionizing radiation, or a genotoxic chemical - to induce DNA damage in the encapsulated cells. For calibration purposes, hydrogen peroxide is generally used to provide a standardized level of DNA damage. The slides are then immersed in a solution that causes the cells to lyse. After lysis of the cells (typically 1 to 2 hours at 4 °C) the slides were washed in deionised water to remove all salts and immersed in an electrophoresis solution. Then electrophoresis was done in 20 minutes by applying electric field and then the slides were neutralized to pH 7 and then it was ready analyzed. This comet assay is used to determine the degree of fragmentation of sperm cells. This assay is also used as a tool for diagnosis of male infertility.

Micromachining is a technology that was used for chemical analysis systems on glass chips by using electroosmotic pumping to drive fluid flow and electrophoretic separation so as to distinguish the samples [9]. Here capillaries of length 1 to 10 centimeters are etched in a glass so that it allows capillary electrophoresis based separations of amino acid up to 75000 theoretical plates in about 15 seconds.

2.4Objectives:

The objectives of the current project is to design a micro slide based gel electrophoresis system by

1. Fabricating individual cartridges for housing the micro slides
2. Constructing a multi chamber system for embedding the cartridges and separate the buffer systems

CHAPTER 3

MATERIALS AND METHODS

SOFTWARES:

1. SolidWorks 2012

MATERIALS:

1. T75 Tissue culture flask
2. Plastic box
3. 35mm Petridish
4. 50ml Conical centrifuge tube
5. Microscope slide
6. Plastic spacer
7. Rubber gasket
8. A pair of syringe
9. Copper wire
10. Cutting tools
11. Sandpapers

CHEMICALS REQUIRED:

1. 0.5M Tris-HCl (pH-6.8)
2. 1.5M Tris (pH-8.8)
3. SDS
4. Acrylamide/Bisacrylamide
5. Ammonium persulfate
6. TEMED
7. Beta mercaptoethanol
8. Glycerol
9. Bromophenol blue
10. Glycine
11. Distilled water

12. BSA (Bovine serum albumin for sample preparation)

13. Protein marker

INSTRUMENTS REQUIRED:

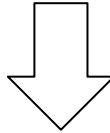
1. Power supply
2. Rocker shaker machine
3. Spirit lamp

METHOD:

Work progress flow chart

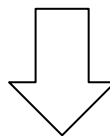
3-D model generation of SDS PAGE device in CAD

- Designing compartments for casting and running in microscope slide.
- Designing compartments for upper and lower buffer



Fabrication of the SDS PAGE device

- Casting compartment
- Lower buffer tank
- Upper buffer tank
- Electrodes in upper and lower tank



Check the model is working or not. If not then optimize the device to work.

3.1 COMPUTER-AIDED DESIGN OF SDS PAGE DEVICE:

All designs were made using the Solid works 2012. Listed below are the components present in the design:

- I. Bottom part
- II. Middle part
- III. Upper part

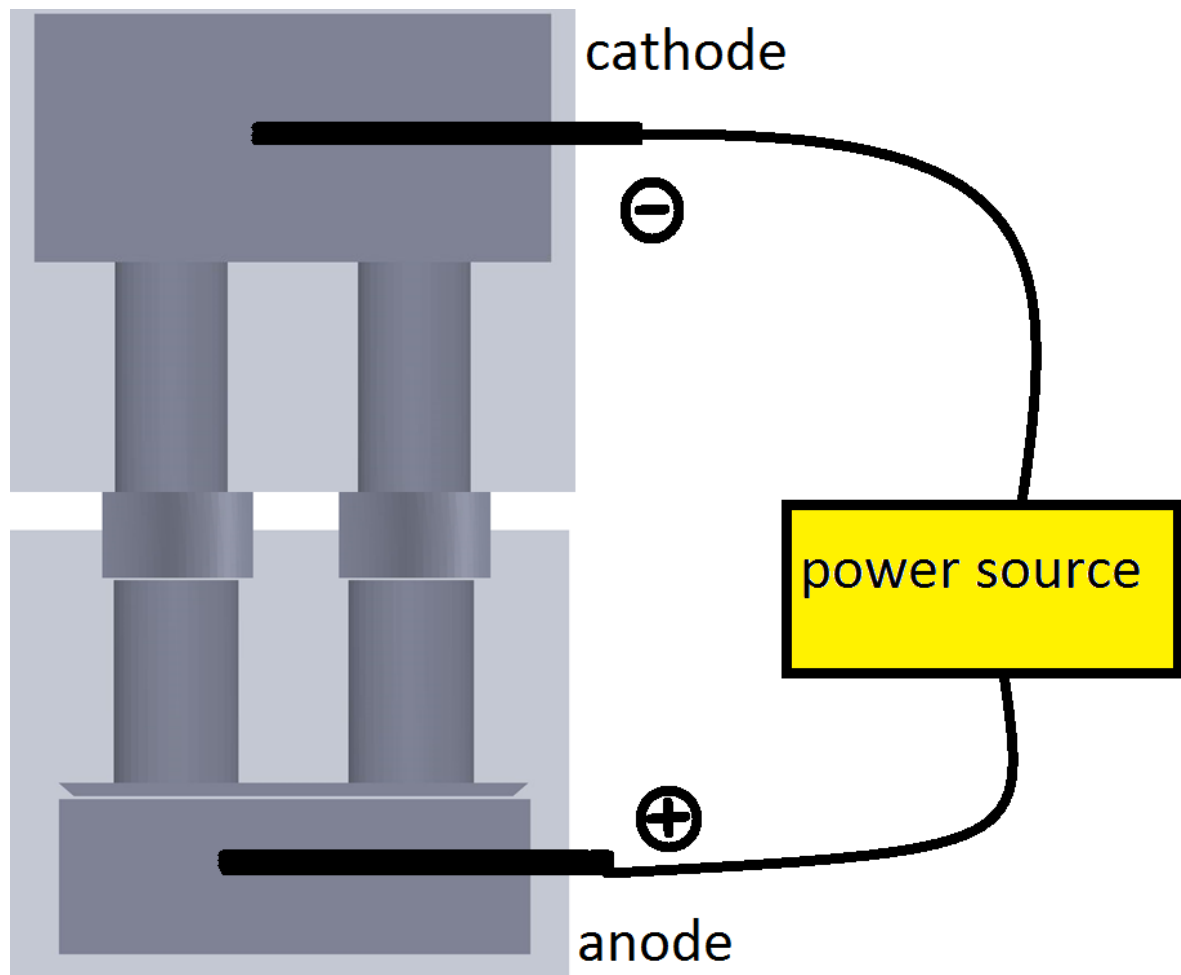


FIGURE 3.1:Assembled SDS PAGE device connected to a power source

THE REACTOR COMPONENTS IN BRIEF:

BOTTOM PART:

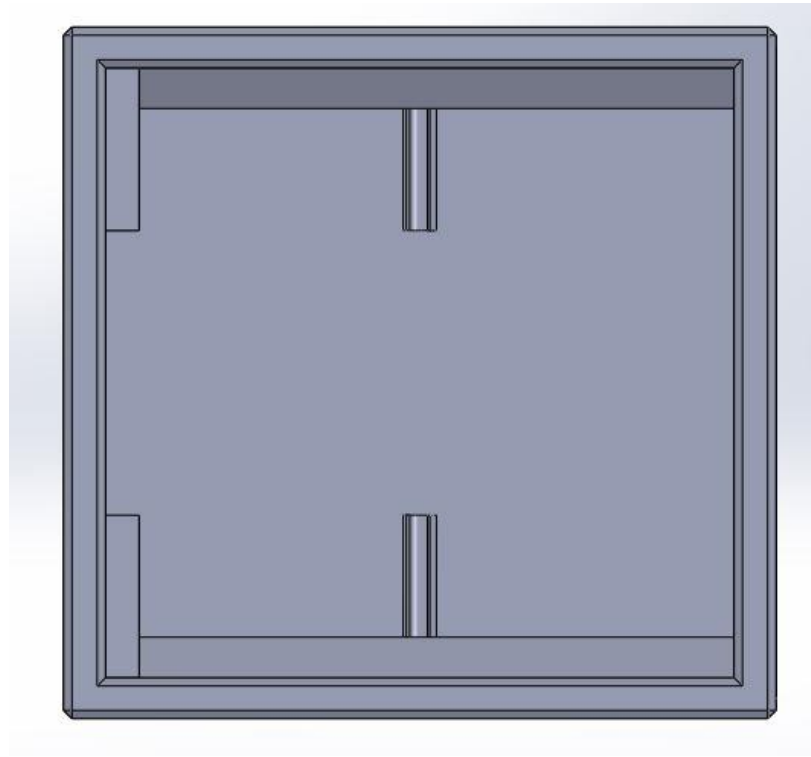


FIGURE 3.2: Top view of the lower buffer tank



FIGURE 3.3:Front view of lower buffer tank

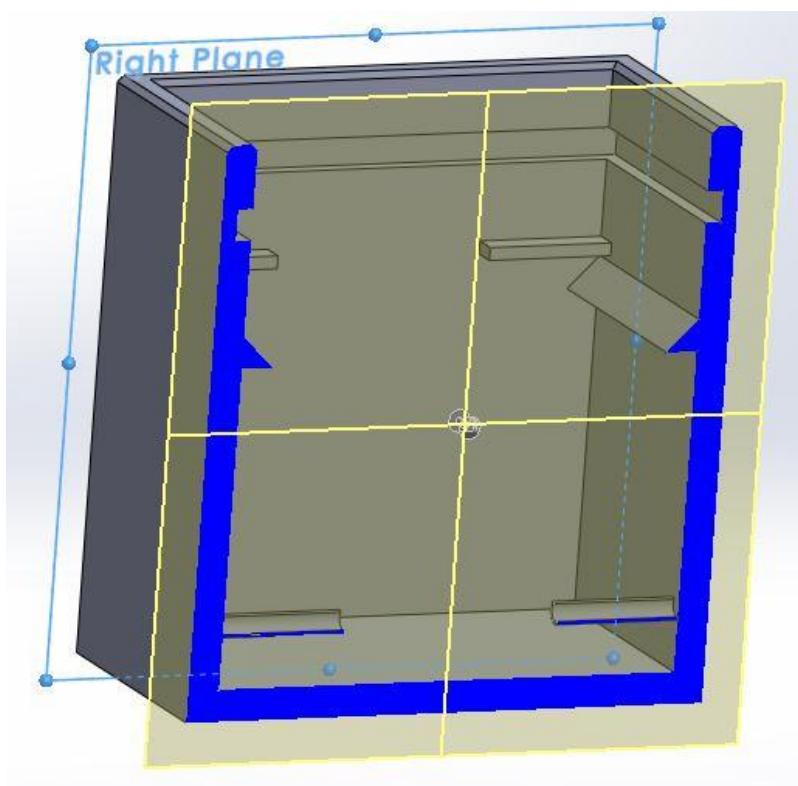


FIGURE 3.4:Cross-sectional view of lower buffer tank

Bottom part of the compartment was solely made to contain a buffer and is used for casting of gel. After microscope slides with spacer were inserted into the middle compartment of the model, stacking gel and separating gel were cast. The bottom part of the middle part contains a gap of width 1mm and a length of 25mm. The gel which we were casting might come out through this gap, hence before casting the gel a plate was placed so that gel would be stopped from coming out and the gels was casted. The slanting part that was extruded from the both sides of the box was for holding and sliding purpose. The slanting was done at the angle of 45°.

FEATURES:

Dimension –8.5 cm X 8.5 cm X 9cm (l X b X h)

Thickness- 0.5 cm

Specialty- Slanting extrusion with slanting angle 45° to hold a plate during casting and hold the middle part during running gel.

RESTING PLATE:

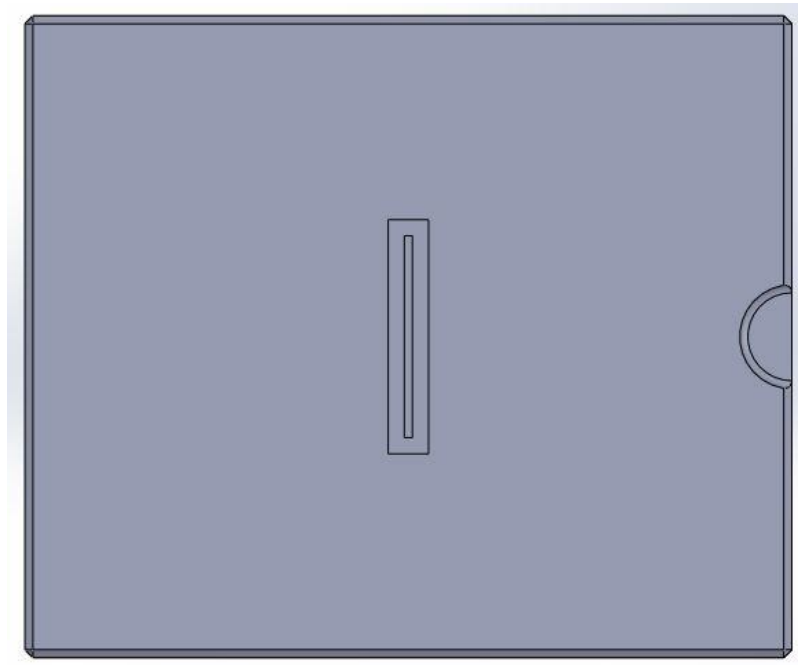


FIGURE 3.5:Cross-sectional view of lower buffer tank

This plate is used for the middle part of the model containing micro-scope glass slide to rest over it for the casting of the gel.

MIDDLE PART:

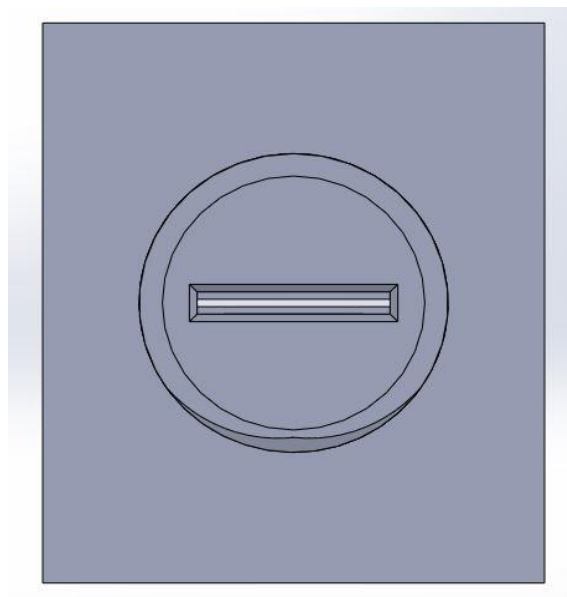


FIGURE 3.6:Top view of middle part

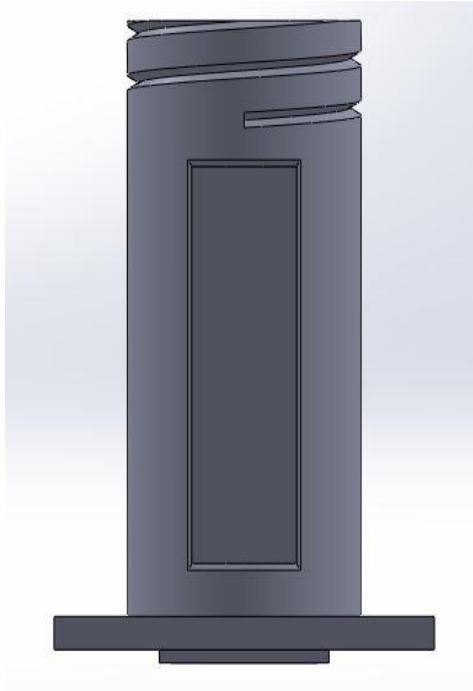


FIGURE 3.7:Front view of middle part

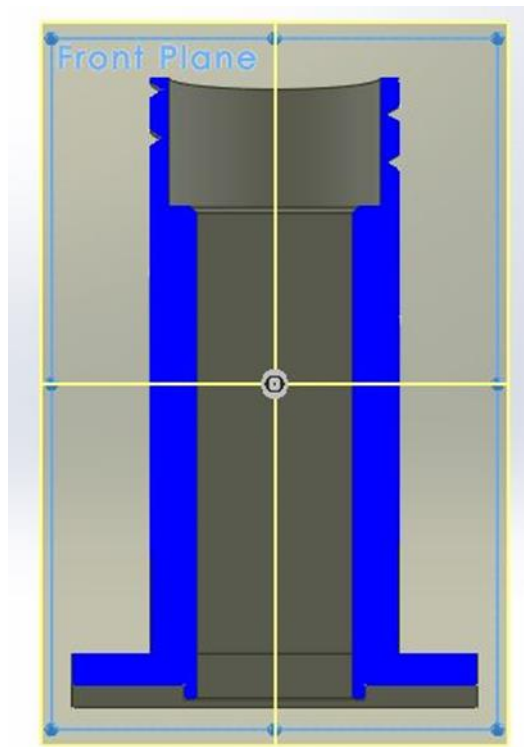


FIGURE 3.8:Cross-sectional view of middle part

This part contains a cylindrical tower. In this tower microscope slides with spacer was inserted and the gel would be cast. These cylindrical towers contain window for observation and analyzation.

FEATURES:

Dimension- Outer cylinder diameter- 4 cm

Inner cylinder diameter-3.4cm (up to 2cm)

Compartment- 2.5 X 0.3 X 7.5 cm (l X b X h)

Window surface -6 X 1.8 cm

UPPER PART:

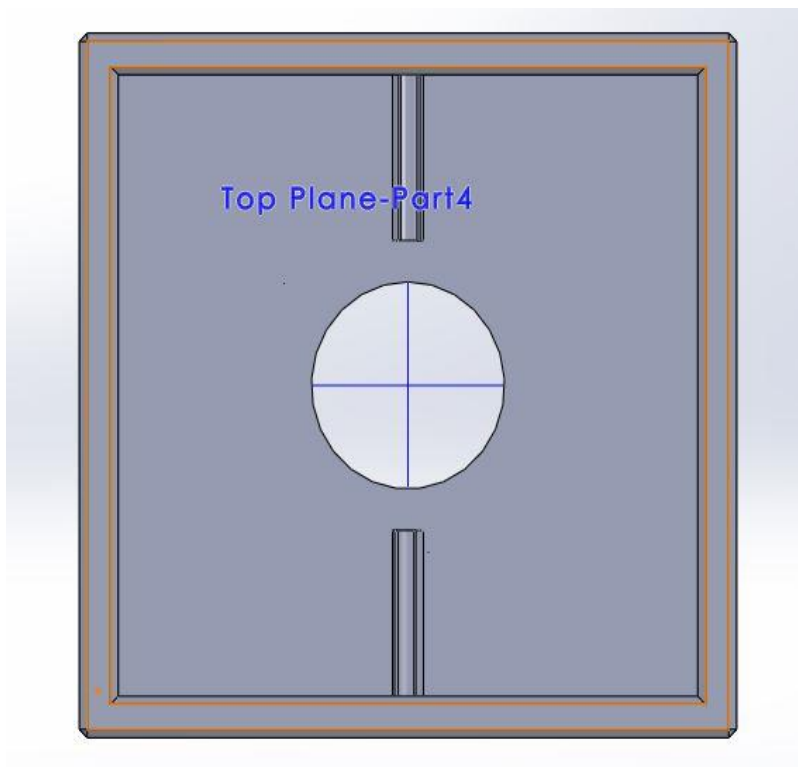


FIGURE 3.9:Cross-sectional view of upper part

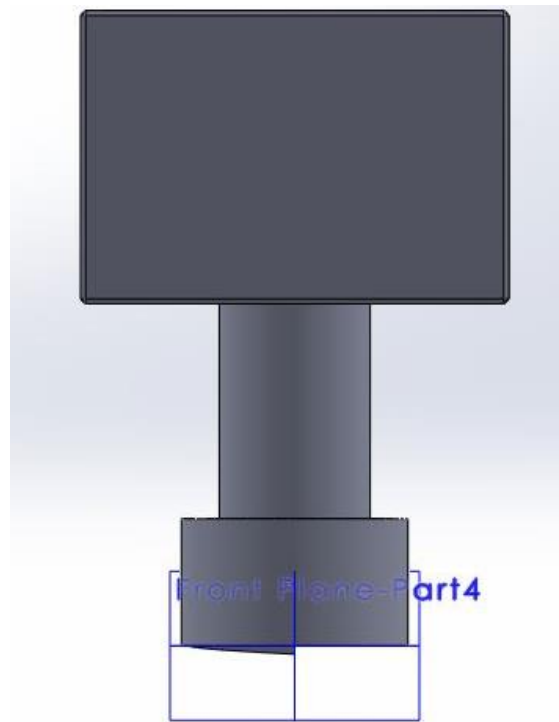


FIGURE 3.10:Cross-sectional view of upper part

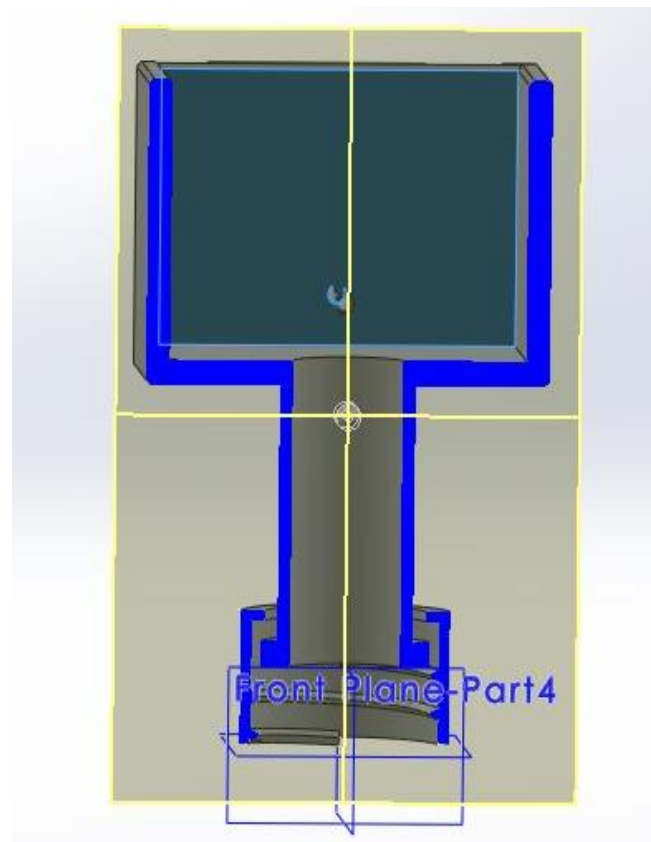


FIGURE 3.11:Cross-sectional view of upper part

The top part of the model contains a buffer tank, delivery tubes and caps. The buffer tank contains the buffer. The delivery tubes deliver buffer from above buffer tank to the gels in middle part of the model containing the microscope and the spacer. A cap was present on the bottom of this part and which can move in circular motion freely. This cap is just like the bottle cap that prevents buffer from leaking and holds the middle and top part firmly. The end of the delivery tube has a larger outer diameter which prevents the cap from coming out.

FEATURES:

Dimension- upper buffer tank- - 16 cm X 16 cm X 8.5cm (l X b X h)

Cylinder outer radius- 3.00 cm

Cylinder inner radius- 2.5cm

Extruded end radius- 3.7cm (thickness 0.5cm)

Cap inner radius- 4.05cm

Cap outer radius- 4.5cm

TOP COVER:

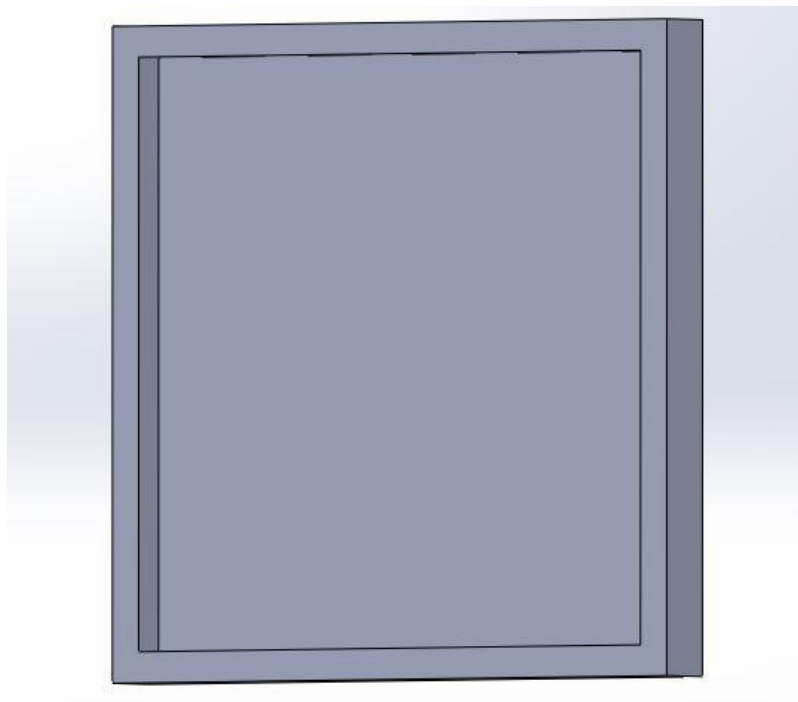


FIGURE 3.12:oblique view of top cover

This top cover is used to cover the upper buffer tank.

MULTISLIDE DESIGNING:

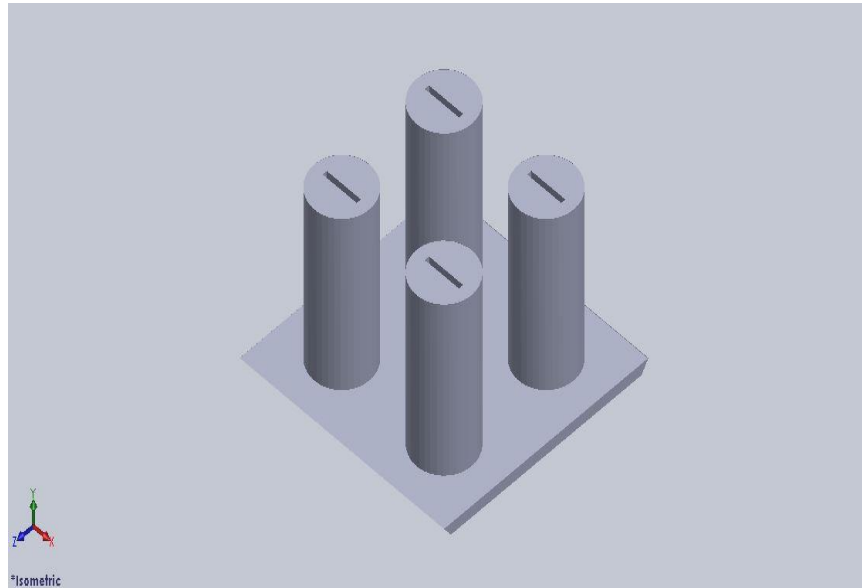


FIGURE 3.13: oblique view of top cover

In the

3.2Fabrication of SDS PAGE device:

Procedure:

Step1: Cut the 50ml centrifuge test tube nearly equal to the height of the glass slide.



FIGURE 3.14:50ml conical test tube before and after cutting

Step2: Cut the 35mm petri dish so that the slide with spacer could be inserted inside through it.



FIGURE 3.15:35mm petri dish

Step3:Now the plastic box for the lower buffer tank was cut as shown. So that's four cuts conical centrifuge test tube would be inserted into the box through the upper cover of it.



FIGURE 3.16:Circular cut on the cover of the lower buffer tank to insert the tubes

Step4:Again the 175 cm^2 tissue culture flask was cut. Its top portion was removed then it was placed over the cut surface of the plastic box and the size of the holes was marked and was cut.



FIGURE 3.17: T75cm² tissue culture flasks

Step5: Then the flask portion was removed, leaving the rectangular part. Again, another t75 cm² tissue culture flask was cut from the back.



FIGURE 3.18: T75cm² tissue culture flask pasted on top of the lower buffer tank

Step6: Now all the cut surfaces were smoothened then conical centrifuge tubes were inserted through the t75 cm² tissue culture flask then through the plastic box.

Step7:The height of the buffer in the upper and the lower buffer tank was decided and marked so that there will be a flow of charges from upper buffer tank to lower.

Step8:As per the height of the buffer the conical centrifuge tubes were attached with the help of silica gel.

Step9:Now the cut petri dish was pasted over the conical centrifuge tube such that the slides would be inserted through it.



FIGURE 3.19:SDS PAGE devicefabricatedwithout electrodes

3.3 Slide preparation:

1. Cut a uniform spacer off into three parts, two for blocking the sides and one to the bottom block.
2. After that a comb is well prepared for casting. While making the comb it should be remembered that the space distribution should be equal.
3. After cutting the spacers rub its surface over sand paper to make it smooth.

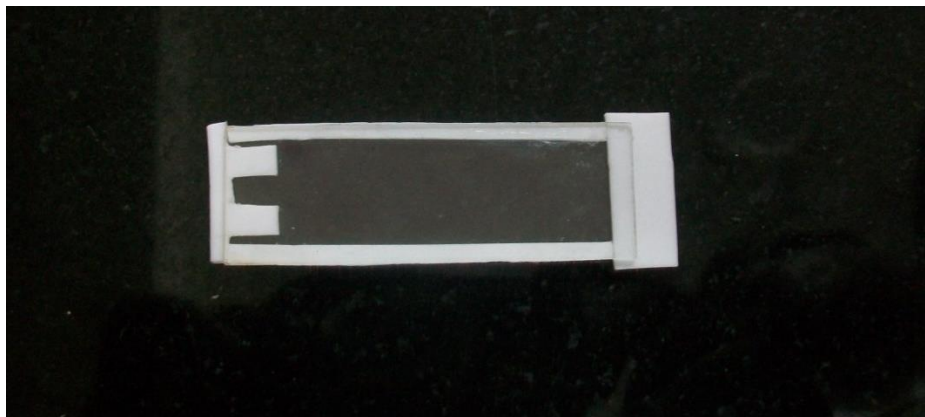


FIGURE 3.20:Glass slide with spacers and comb

3.4 SDS PAGE protocol used:

TABLE1:Resolving gel preparation

| Components | Volume 10ml resolving gel solution |
|---|------------------------------------|
| | For 10% gel |
| Deionized | 4.13 ml |
| 30% acylamide/bisacrylamide | 3.3 ml |
| 1.5 Tris-HCl containing 0.4%SDS, pH 8.8 | 2.5 ml |
| 10% APS | 60 μ l |
| TEMED | 13 μ l |

TABLE2:Stacking gel preparation

| Components | Volume 5ml stacking gel solution |
|--------------------------------|----------------------------------|
| | |
| Deionized | 2.975 ml |
| 30% acylamide/bisacrylamide | 0.67 ml |
| 0.5 Tris-HCl containing pH 6.8 | 1.25 ml |
| 0.4%SDS | 0.05ml |
| 10% APS | 0.05ml |
| TEMED | 0.005ml |

TABLE3:5X Sample buffer

| | |
|-----------|---------------------|
| 10% w/v | SDS |
| 10 mM | Betamercaptoethanol |
| 20% v/v | Glycerol |
| 0.2 M | Tris-HCl, pH-6.8 |
| 0.05% w/v | Bromophenolblue |

TABLE4:1X Running buffer

| | |
|------------|----------|
| 25 mM | Tris-HCl |
| 200 mM | Glycine |
| 0.1% (w/v) | SDS |

3.5 Procedure:

1. Separating gel was made as described above, then was poured into the glass slides whose three sides were blocked by a spacer and the gel was poured from the top with the help of a micropipette.
2. Vaseline was applied so that there will be no leakage. Then gelate formation was started which took 20- 30 minutes.
3. Then stacking gel was made and was poured over the separating gel until it was overflowing.
4. Then comb was inserted into the stacking gel to form wells. While inserting the comb it was taken care that there was no air bubble in between. After that it took 20- 30 minutes to gelate.
5. The sample was prepared by mixing a sample with sample buffer and then it was heated in boiling water for 5- 10 minutes.
6. After that prepared sample was loaded into the well carefully without overflow. Then the protein marker was loaded in other well.

Whole setup:



FIGURE 3.20: Whole setup of SDS PAGE

1. Upper buffer tank was connected to anode and lower buffer tank with cathode. Voltage was set.
Applied voltage- 30 volt
Current-4- mA
Time- 6 hours (approximately)
2. The process was completed when the protein marker reached the bottom. The power supply was stopped and the slides were taken out.
3. Gel was recovered then stained in coomassie blue then destained.

TABLE5:Gel staining solution

| | |
|-----------------------|--------|
| Methanol | 400 |
| Acetic Acid (glacial) | 100 ml |
| H ₂ O | 500 ml |
| Coomassie blue R-250 | 1 g |

TABLE6:Gel destaining solution

| | |
|-----------------------|--------|
| Methanol | 400 |
| Acetic Acid (glacial) | 100 ml |
| H ₂ O | 500 ml |

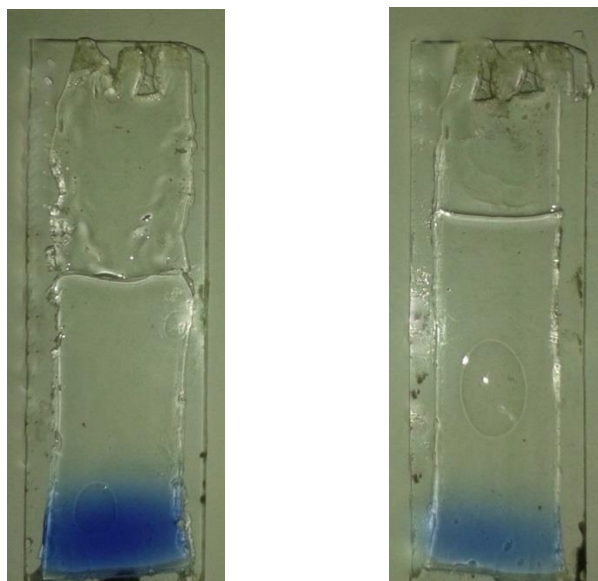


FIGURE 3.21: stained gels

CHAPTER 4

RESULTS & DISCUSSION

The SDS page device that was designed is completely leak proof because of the following reasons:

1. The connection between the upper buffer tank and the lower buffer tank is through the middle cylindrical extruded part. The cap that is present on the upper part tightly closes the cylinder like a bottle cap closes the mouth of the bottle.
2. We will use rubber spacers which will be inserted along with two microscope slides. The rubber spacer will push the glass slides outwards towards the wall.

Like currently available SDS PAGE device, it is a multi-chamber. At a time we can use n number of microscope slides. Also we can design this device for as many samples we want to run simultaneously.

The in-house developed electrophoresis device was validated by running SDS PAGE. The prototype protein was BSA (Bovine Serum Albumin). The protein marker was Genei (manufacture in Banglore, India) marker range was from 29 to 205 kD. After staining the prepared gel with staining solution and then again destaining it to visualize, we got the following results as shown below. The molecular weight of BSA comes in between 62 to 66.5 kD. Genei unstained protein molecular marker was used which shows best result in the range between 29 to 205 kD. Since BSA lies in that range we would be able to see the protein band of it. Exact molecular weight of any protein within this range can be calculated by comparing the band results from the molecular marker.

In the below figure there is some mixing between protein ladder and BSA sample. This might be due to some handling error while loading the BSA or the protein molecular marker. While moving through the gel both the protein marker and BSA sample was mixed and we can visualize multiple bands in the range of 205 to 29 kD instead showing band in the range 60-66.5.

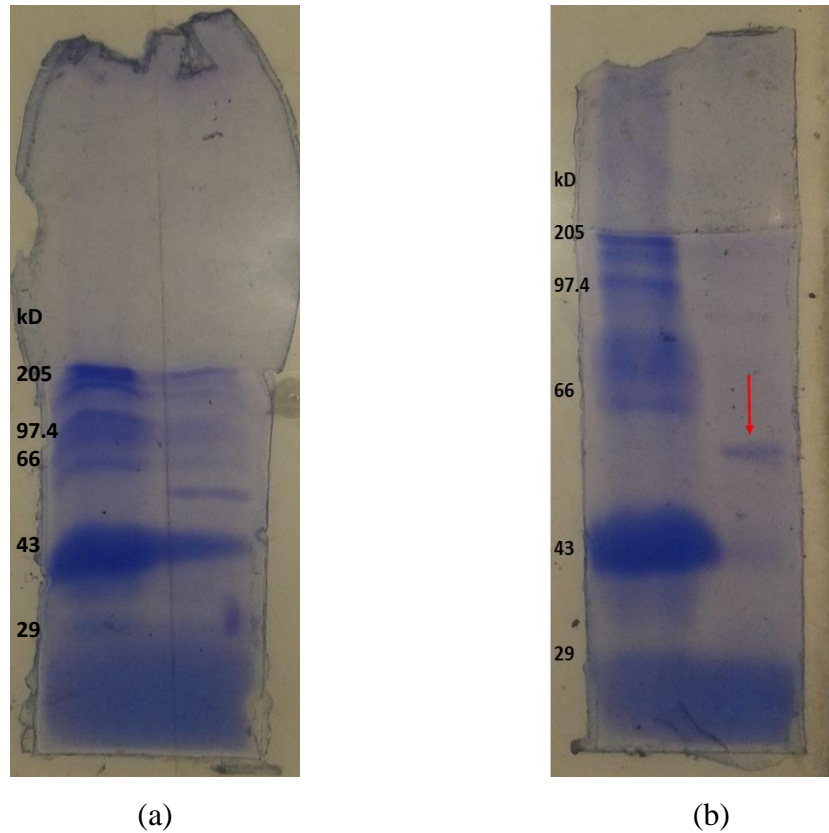


FIGURE 4.1: Bands present in the left side shows protein molecular marker and the bands in the right side shows the bands of BSA sample

In the figure (b) it can be said by comparing the bands in the Genei molecular marker it shows the protein band for BSA in the range of 60-66.5 kD which is correct.

CHAPTER 5

CONCLUSION & FUTURE WORK

CONCLUSION:

Hence a cost effective SDS PAGE was designed by incorporating microscope slide. As per the design the microscope slide can be housed in the cylindrical cartridges embedded in a multi chamber system. With 3-Dimensional modelling of the system shows the buffers for the both ends (polar ends i.e. Cathode and anode) of the electrophoretic system can be effectively separated.

In the classical method of SDS PAGE, the volume of acrylamide gel will be $\approx 5\text{ml}$ and stacking gel is $\approx 2\text{-}3\text{ml}$ and the minimum amounts of sample running well is not less than 7. So its always require to run a sample in equal amount in all 7 or more wells to get a clarify results. To say usually works are done targeting a specific protein and enzyme. So to have a continuous assessment of enzyme or protein under the study SDS PAGE is vital. And the major drawback of classical method is wastage of chemicals and samples under study.

To overcome disadvantages a new multi slide SDS PAGE set up can be used for effective protein analysis as similar to classical method and economical than classical method. And here the volume of separating gel will be $\approx 1\text{ml}$ and the stacking gel will be $\approx 0.5\text{ ml}$. another advantage of multi slide SDS PAGE system used in present study has a provision to run four SDS slides of each slide having sample loading of two wells. And so for single protein at a time four different concentration of SDS PAGE can be done to confirm the optimum percentage of gel to be used for the specific protein sample.

FUTURE WORK:

The three dimensional designed model has to be fabricated as mentioned. Then it has to be run for different proteins and the results should be compared with the results obtained from the standard page device available in the market. The voltage and current required

for this much smaller amount of protein sample has to be optimized. The amount of different chemicals should be taken in such a small volume is to be calculated.

The handmade model that has been fabricated can be optimized by optimizing different heights of the slide holder then running the gel. Furthermore thickness of gel can be varied and the result of SDS PAGE can be compared and optimized.

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